DEVICE AND METHOD FOR FOCUSING SOLUTES IN AN ELECTRIC FIELD GRADIENT

CROSS-REFERENCE TO RELATED APPLICATION

The present application claims the benefit under 35 U.S.C. 119(e) of the priority of the filing date of copending U.S. provisional application Serial No. 60/084,505, filed May 6, 1998, which is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to an electrophoretic device and method and, more particularly to an electrophoretic device and method that establishes and maintains an electric field gradient using an electrode array in which the electrode voltage is individually controlled.

BACKGROUND OF THE INVENTION

Electrophoresis is a gentle, inexpensive method of separating molecules based on their movement in an electric field. Electrophoresis can be carried out in free solution, e.g., an open capillary, slit or annulus, or with the aid of a support medium, such as a gel, polymer solution, or granular packing. Electrophoresis requires a buffered electrolyte to maintain the required pH and provide sufficient conductivity to allow the passage of current.

More than a decade ago, O'Farrell described a method known as counteracting chromatographic electrophoresis (CACE) in which proteins could be focused at the interface between two different gel filtration media packed into the upper and lower halves of an electrochromatography column. *Science* 1985, 227, 1586–1588. The results were soon replicated by others who found that at least one protein, ferritin, could be concentrated beyond 100 mg/mL. *Sep. Sci. Technol.* 1988, 23, 875; *Sep. Purif: Methods* 1989, 18, 1. This remarkable feat was tempered by the finding that his approach worked poorly with protein mixtures and would be difficult to scale up. *Biotechnol. Prog.* 1990, 6, 21. Nevertheless, O'Farrell had found a way to focus proteins in an electric field that did not require the use of a pH gradient.

CACE is only one member of a family of electrophoretic focusing techniques which can be described by the simple flux equation,

$$N_{p,x} = -D_p \frac{dc_p}{dx} + \left(\langle u_{p,x}\rangle + z_p \omega_p \frac{I_x}{\sigma}\right) c_p = 0 \qquad (1)$$

where $N_{p,x}$, the molar flux of protein along the x-axis, is set equal to zero for stationary, focused protein bands. Eq.(1) is composed of a dispersive term, a convective term and an electrophoretic term where c is the protein concentration, D_p is a diffusion or dispersion coefficient, $\langle u_{p,x}\rangle$ is the apparent chromatographic protein velocity along the x-axis, z_p is the protein charge, ω_p is the protein mobility, I_x is the current density and σ is the electrical conductivity. In order for proteins to focus it is necessary that at least one of the terms in parentheses vary so that their sum (1) forms a gradient in which (2) vanishes at a single point in the chamber. Focusing occurs at the point in the chamber where the gradient vanishes.

Setting the sum of the terms in parentheses in eq.(1) equal to zero, it is seen that focusing may be accomplished in at least five different ways: (1) in a pH gradient with u_p=0, proteins will focus at the point where the net charge on the protein vanishes, i.e., z_p=0, as is the case with isoelectric focusing (IEF); (2) in a gradient in $u_{p,x}$ with z_p, I and σ held constant, which corresponds to CACE; (3) in a gradient in $\omega_p$with $u_{p,x}$ z_p, I and σ constant, e.g., focusing a protein in a urea gradient, a technique which is still untested. With u_p held constant there are still two ways left to focus proteins: by forming gradients in I or σ, both of which generate gradients in the electric field.

Recently, Koegler and Ivory demonstrated that charged proteins could be separated and focused using an electric field gradient in an electrochromatography column. *J Chromatogr.*, A 1996, 229, 229–236. A fluted cooling jacket was used to form a linear gradient in the electric field which drove the proteins against a constant flow of buffer in a packed dialysis tube. This approach was slow and cumbersome and gave mediocre results, but it successfully illustrated an alternative focusing technique known as electric field gradient focusing (EFGF).

Next, Greenlee and Ivory showed that proteins would focus in the electric field gradient formed by an axial conductivity gradient and opposed by a constant flow of buffer. *Biotechnol Prog.* 1998, 14, 300–309. Greenlee's apparatus was far simpler to build and operate than was Koegler's. The device was also surprisingly fast when run in free solution, reaching equilibrium in less than 10 min., and gave unexpectedly good results when filled with a 40-μm size exclusion (SEC) packing.

Focusing can also be achieved by opposing a constant convective velocity with a gradient in the electrophoretic velocity of the protein. This gradient can be created by varying the net charge on the protein (as in isoelectric focusing), by varying the cross-sectional area through which the electric current travels, as with electric field gradient focusing, or by varying the buffer conductivity.

Isoelectric focusing (IEF) is a gradient focusing method which varies the charge on a protein using a pH gradient. The convective velocity is usually set to zero while the net charge on the protein decreases as it approaches its isoelectric point (pI). The protein focuses at this point since its net charge, and therefore its electrophoretic velocity, both vanish at its pI.

Conventional IEF is usually performed in a support medium such as agarose or polyacrylamide gel. The pH gradient is formed by using a complex set of reagents known as carrier ampholytes which generate a stable, linear pH gradient under the influence of an applied electric field. Proteins migrate to the region where the ampholyte solution pH is equal to its own pI. In gels, detection of the focused bands involves a time consuming stain/destain procedure, and the ampholytes should be removed before the stain is applied. Established IEF protocols and a succinct history of its development are given by Righetti (1983).

Despite the advances in the electrophoretic methods and devices noted above, a need exists for electrophoretic methods and devices that can effectively separate charged solutes, such as protein mixtures, into their component solutes. The present invention seeks to fulfill these needs and provides further related advantages.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides an electrophoretic device for focusing a charged solute. The device includes a first chamber for receiving a fluid medium, the first chamber having an inlet for introducing a first liquid to the chamber and an outlet for exiting the first liquid from the chamber; a second chamber comprising an electrode array, the second chamber having an inlet for introducing a second